Abstract No. stav484

Crystal Structure of the FHA Domain of the Chfr Mitotic Checkpoint Protein

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Beamline(s): X9A

Introduction: Chfr was identified as a checkpoint protein that arrests cell cycle progression in response to mitotic stress. The gene that encodes Chfr is frequently inactivated in cancer cell lines, yet the function of the protein is poorly understood. Chfr has ubiquitin ligase activity in vitro, which is mediated by its RING finger domain. At its N-terminus, Chfr has an FHA domain, which may recruit substrates for its ubiquitin ligase activity. To obtain insights regarding the function of Chfr, we solved the structure of its FHA domain and compared it to the previously determined structures of the Rad53 N- and C-terminal FHA domains bound to phosphothreonine and phosphotyrosine-containing peptides, respectively.

Methods and Materials: The human Chfr FHA domain (residues 14-124 or 14-128) was expressed in E. coli BL21 cells. Crystals were grown by the hanging drop vapor diffusion method by mixing the protein with an equal volume of reservoir solution containing 14%-18% polyethylene glycol (PEG) 8000, 100 mM bis-Tris [pH 6.5] and 5 mM DTT. Crystals of the native polypeptide containing residues 14-124 formed in space group P2(1)2(1)2(1) with a= 54.90 Angstroms, b= 52.90 Angstroms, c= 77.25 Angstroms, and contained two molecules in the asymmetric unit. Crystals of selenomethionine (SeMet)-labeled polypeptide containing residues 14-128 formed in space group P3(2)21 with a=b= 62.36 Angstroms, c= 54.25 Angstroms, and contained one molecule in the asymmetric unit. Some of these SeMet-labeled crystals were soaked in harvest buffer (21% PEG 8000, 100 mM bis-Tris [pH 6.5], 75 mM KCl) supplemented with 0.5 mM mercury chloride and 1 mM b-mercaptoethanol for 1h. All data sets were collected using flash-frozen crystals. The native P2(1)2(1)2(1) data set was collected at the F1 beamline of the Cornell High Energy Synchrotron Source (MacCHESS); the selenomethionine and selenomethionine-mercury chloride derivative data sets were collected at the X9A beamline of the Brookhaven National Laboratory (BNL) Synchrotron Source. Reflection data were indexed, integrated and scaled using programs DENZO and SCALEPACK. Phases for the P3(2)21 space group were calculated using the isomorphous signal of the Hg-SeMet derivative and the anomalous MAD data collected from the SeMet crystals using the program MLPHARE. The model was built using the program O and refined with the program CNS. A partially refined model in the P3(2)21 space group was used to solve the structure in the P2(1)2(1)2(1) space group by molecular replacement using the program AMORE. The P2(1)2(1)2(1) model was refined using the programs O and CNS.

Results: The Chfr FHA domain adopted a beta-sandwich fold. The region that binds the phosphopeptides in the Rad53 domains was structurally conserved in Chfr, suggesting that Chfr also recognizes phosphopeptides. Adjacent to the residues predicted to recognize phosphopeptides, Chfr has a cluster of buried charged and polar residues that is conserved in the N-terminal, but not in the C-terminal FHA domain of Rad53. The most surprising feature of the Chfr FHA structure was that the domain crystallized as a domain-swapped dimer, unlike the Rad53 FHA domains, which were monomeric.

Conclusions: FHA domains can be divided into families that can be distinguished by the presence of buried conserved polar and charged residues adjacent to the residues that directly contact ligand. These different families of FHA domains may have distinct binding specificities. In addition, FHA domains may have different tendencies to dimerize, as suggested by the unexpected observation of Chfr crystallizing as a domain-swapped dimer. The structural features of the Chfr FHA domain will guide further efforts to probe the function of Chfr in vivo.

Acknowledgments: The authors thank Steven Smerdon for providing the coordinates of the Rad53 N-FHA domain before publication, the Wistar Institute Protein Chemistry and Nucleic Acid Facilities for protein N-terminal sequencing, mass spectrometry analysis and DNA sequencing analysis, the staff of the Brookhaven National Laboratory and Cornell High Energy Synchrotron Source for help with data collection and David Speicher, Ronen Marmorstein and Roger Burnett for support and helpful discussions. Funded by grant CA89630 from the National Cancer Institute.